# Kazuko M. HASEGAWA*: Cytotaxonomic notes on *Cimicifuga foetida* from Bhutan**

## 長谷川一子*: ブータン産コウライショウマの細胞分類学的知見**

Three living plants of *Cimicifuga foetida* L. were brought back from Bhutan by the members of the third Botanical Expedition to Eastern Himalaya by the University of Tokyo in 1967. I have been working on Asiatic species of *Cimicifuga* for these few years, and now I had a chance to study about the living plants of *C. foetida* for the first time. This species is distributed in three separate areas of Asia, and also in the middle part of Europe (Fig. 1).

The chromosome numbers of all the species of *Cimicifuga* including *C. foetida* hitherto known are 2n=16, and are considered to be diploid. The chromosomes of the plants of Bhutan are, however, found to be 2n=32, and are considered to be tetraploid. In the present study, I have tried to define the relation between diploid and tetraploid plants of *C. foetida* by means of the examination of herbarium specimens.

The three plants were collected from Thimphu, Bhutan at 2450 m alt. by Dr. H. Kanai and Mr. H. Ohashi on April of 1967. Two of them have been cultivated in the experimental garden of the University of Tokyo, and the other one in Karuizawa. In last summer (1968), young buds were grown on both plants in Tokyo, but died before flower, so the flowers of them have been unable to observe.

**Cimicifuga foetida** L. in Syst. Nat. ed. 12: 659 (1767).

Herbaceous stinking perennial. Stem erect 1–1.5 m tall, slightly striated, glabrous or sparsely pubescent. Both radical and cauline leaves bi- or triternate sometimes pinnate-tri-ternate. Leaflet glabrous or slightly pubescent rarely densely pubescent, 4–10 cm long, 2–5 cm wide, ovate or lanceolate-oblong, sharply serrate on the margin; acute to acuminate at the apex, shortly attenuate at the base. Inflorescence a lax and elongate terminal

* Department of Botany, Faculty of Science, University of Tokyo, Hongo, Tokyo. 東京大学理学部植物学教室

** 東京大学インド植物調査隊報告 No. 19.

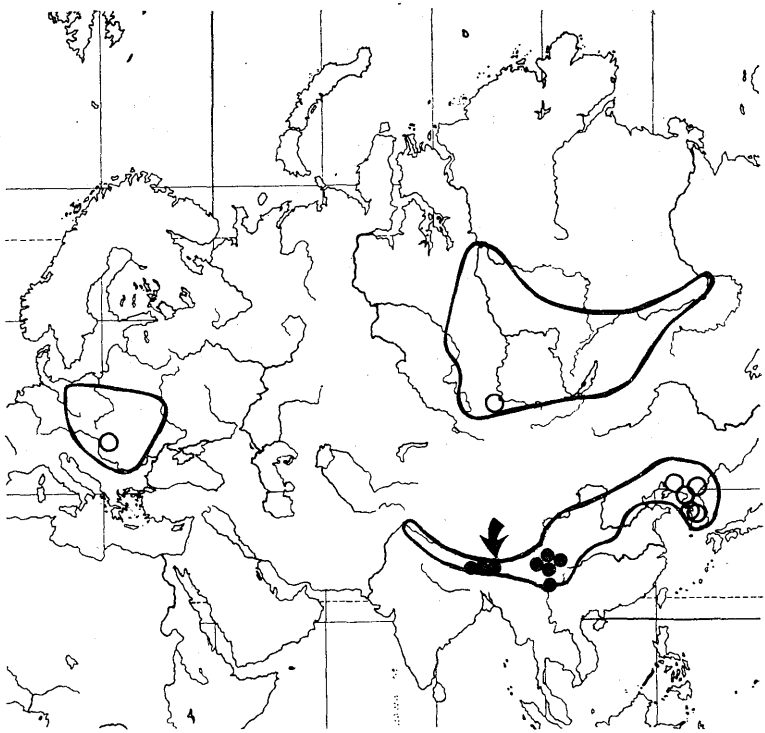

Fig. 1. Distribution map of *C. foetida*. Open circles show the plants considered to be 2n=16; Solid circles show the plant of 2n=32 (arrow), and those considered to be 2n=32.

raceme with many lateral ones. Flowers pedicellate with the pedicel 4 mm long. Sepals 4–6 in a flower, membranaceous, ovate 3–4 mm long, creamy white, caducous. Petals white, ovate 2.5–3 mm long with basal concave nectary; the apex entire or bilobed, often thickish. Carpels three or four, the mature follicle ovate 13–15 mm long, stipitate with the stipe 2–3 mm long; style slender and hooked, 2 mm long. Seeds ovate 3–4 mm long, chaffy as a whole with very thin wings on both sides.

Nom. Jap. Kôrai-Shôma

Distr. Southern Siberia, Manchuria, Mongolia, Korea, northern and western China, Himalayas, and middle Europe. (Fig. 1.)

It was found for the first time that *C. foetida* from Bhutan had 2n=32

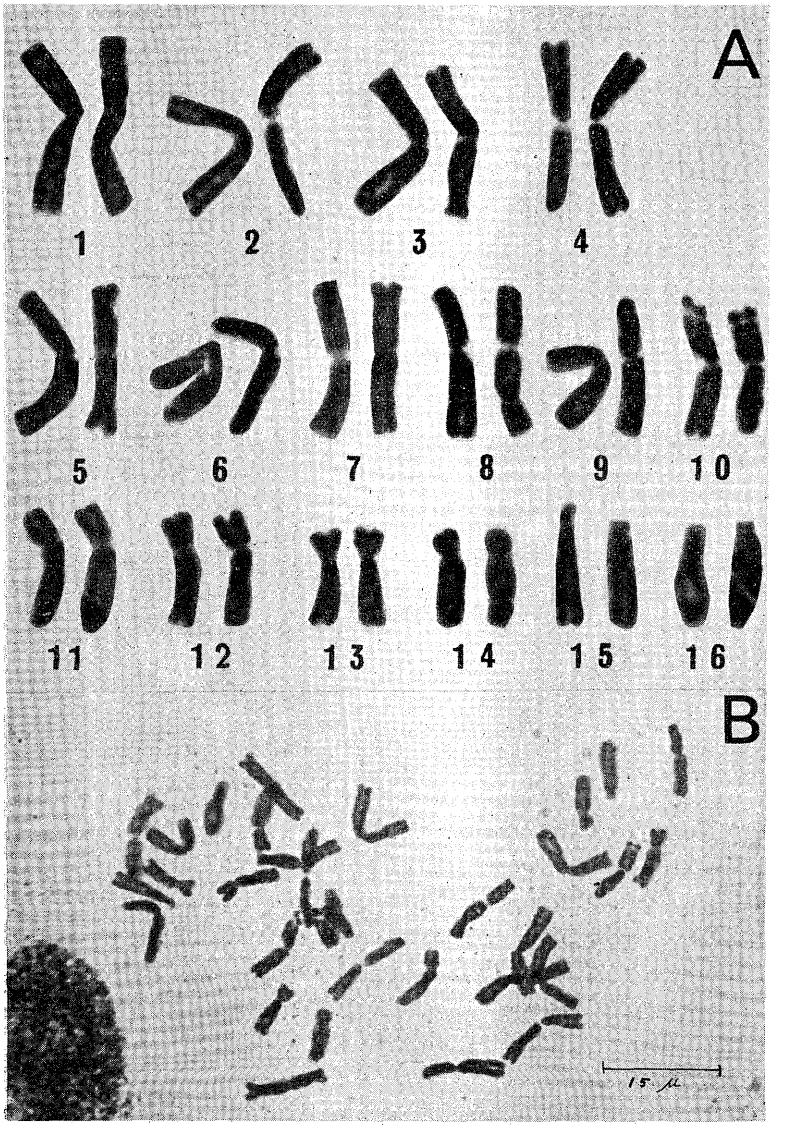


Fig. 2. A & B. Somatic chromosomes of *Cimicifuge foetida* L. from Bhutan.
A. A karyogram produced from B.

chromosomes. The previous authors have reported the species to have 2n= 16 chromosomes (Table 1). It is considered that the latter is diploid and the former is tetraploid judged from the chromosome numbers and karyotypes, which are expressed as follows;

K(2n)=16=10V+4J+2I diploid (judged from the karyogram of Lewitsky)
K(2n)=32=20V+8J+4I tetraploid (Bhutan) (Fig. 2)

A pair of telocentric chromosome with secondary constriction is reported in diploid chromosomes by Lewitsky. A pair of metacentric chromosome with secondary constriction is found in tetraploid chromosomes. The cytological technique applied in this study is a modification of the oxyquinoline-aceto-orcein squash method described in my previous report of Journ. Jap. Bot. 43: 141-155, 1968.

Table 1. Chromosome numbers of *C. foetida* L.

| 2n | n | Locality | Authors |
|---|---|---|---|
| | 8 | | Langlet 1927 |
| 16 | | München, Germany | Langlet 1932 (under the name of *C. simplex*) |
| 16 | | USSR | Lewitsky 1941 |
| 32 | | Thimphu, Bhutan | Hasegawa |

The relation between diploid and tetraploid were presumed from dried specimens. *C. foetida* is morphologically very variable, especially the shape of the apex of petals and the absence or presence of pubescence are remarkable. Those variations were, however, observed throughout the range, and were recognized not to locally occur. Then the size of pollen grains and stomata were comparatively examined.

Pollen grains were observed by the method of Dr. M. Ikuse. Pollen grains taken from dried specimens were transferred onto a slide, washed with xylol and absolute alcohol, and stained with gentian-violet. They were observed after covered with glycerin-jelly. The pollen grains of *C. foetida* are 3-colpate and surface pattern is subreticulate, and no variation were found through all specimens observed in this respect. The size of the pollen grains of 14 specimens (30-60 grains per a specimen) were measured and shown in Table 2 and Fig. 3. The pollen grains of *C. foetida* from Bhutan,

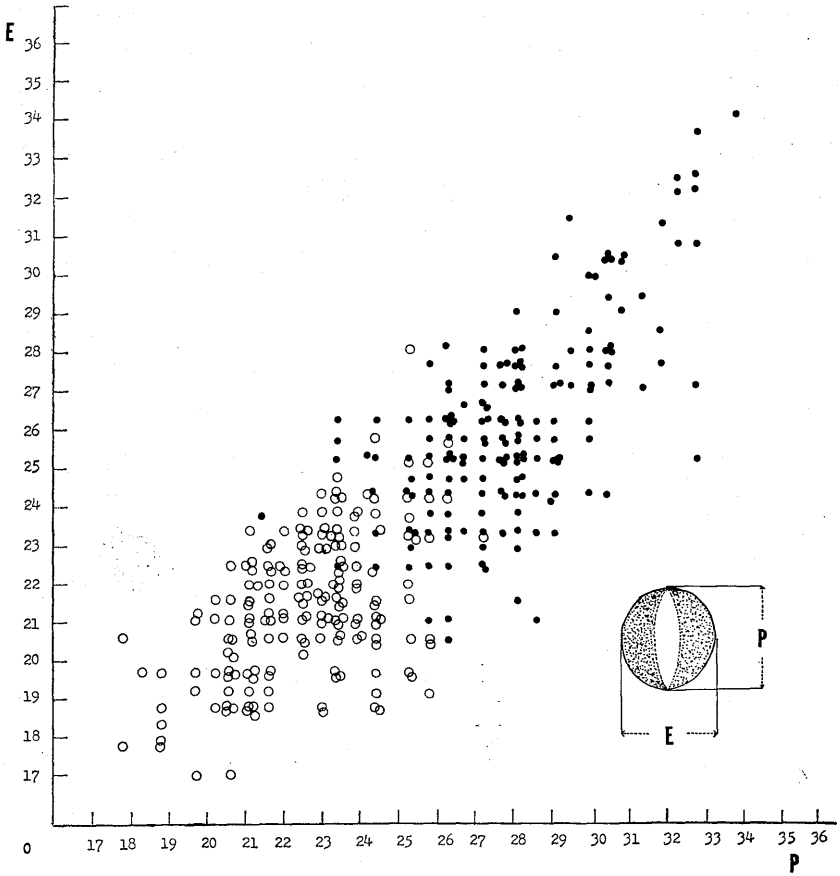


Fig. 3. Scatter diagram illustrating the variation in pollen size. Solid circles show the pollen from Himalaya, Burma and Yunnan, China; open circles show those from Siberia, Manchuria, Korea and Europe. P: Polar diameter. E: Equatorial diameter.

Yunnan, China and Burma are averaged at 27.8 $\mu \times 26.2\ \mu$, on the other hand those from Manchuria, Korea, Altai and Europe are averaged at 22.6 $\mu \times 21.8\ \mu$; obviously the latter is smaller than the former.

For the observation of stomata, the mature leaflets of dried specimens were softened by boiled water except the living leaflet of Bhutan, and only the select stomata which are opened at the same degree were measured. The size of the stomata was also measured about 13 specimens and a living

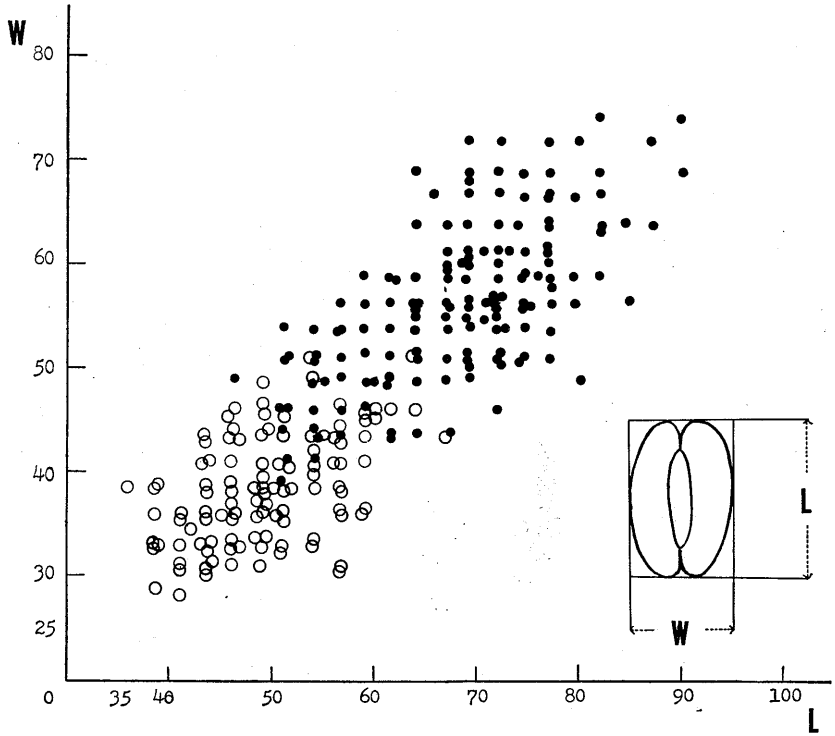


Fig. 4. Scatter diagram illustrating the variation in stomatal size with guard cells. Solid circles show the stomata from Himalaya, Burma, and Yunnan, China; open circles show those from Siberia, Manchuria, Korea and Europe. L: Length ($\mu$). W: Width ($\mu$).

plant of Bhutan (30–60 stomata per a specimen). The results were very similar to those of pollen grains. (Table 2, Fig. 4) The stomata of the plants from Bhutan, Darjeeling, Nepal, Yunnan and Burma are averaged at 67.7 $\mu\times$ 56.8 $\mu$, on the other hand, those from Manchuria, Korea, Altai, and Europe are smaller as 50.3 $\mu\times$39.8 $\mu$ on an average.

It was recognized that the pollen grains and stomata of *C. foetida* are distinctly larger in the area of Nepal, Bhutan, Yunnan, Burma, etc. than those in the area of Altai, Manchuria, Korea, and Europe. It is considered that the size of pollen grains and stomata seem to be correlate with the characters of diploid and tetraploid and that *C. foetida* in northern Asia and Europe may be diploid but that in Himalayas and Yunnan, and a more

Table 2. The size of pollen grains and stomata of *Cimicifuga foetida* L. considered to distinguish diploid and tetraploid.

| Locality (Herbarium) | Pollen | | | Stomata | | |
|---|---|---|---|---|---|---|
| | Numbers examined | P ($\mu$) Average | E ($\mu$) Average | Numbers examined | L ($\mu$) Average | W ($\mu$) Average |
| Chromosome number 2n=32 | | | | | | |
| Bhutan: Thimphu (a living plant) | | | | 60 | 68.6 | 60.4 |
| Chromosome number judged to be 2n=32 | | | | | | |
| Bhutan: Belley La (KYO, No. 587) | 60 | 27.6 | 25.0 | 60 | 71.9 | 57.5 |
| Bhutan: Belley La (KYO, No. 588) | 60 | 28.8 | 26.6 | 60 | 67.4 | 57.0 |
| India: Darjeeling (TI, No. 7777) | | | | 30 | 66.9 | 59.3 |
| Nepal: Diorali Bhanjang (TI) | | | | 30 | 77.4 | 64.9 |
| Burma: Adung Valley (GH, No. 9906) | 30 | 28.7 | 28.0 | 30 | 59.3 | 52.5 |
| China: Yunnan, Soo-roo-la (AA, No. 66723) | 30 | 26.0 | 25.0 | 30 | 69.1 | 53.9 |
| China: Yunnan (AA, No. 6999) | 30 | 27.6 | 25.8 | 30 | 56.3 | 47.5 |
| China: Yunnan, Shi-gi-tung (AA, No. 67203) | 30 | 26.5 | 24.5 | | | |
| China: Yunnan (AA, No. 21111) | 30 | 29.6 | 28.7 | | | |
| China: Yunnan, Chungtien (AA, No. 13480) | | | | 46 | 72.8 | 57.9 |
| Average | | 27.8 | 26.2 | | 67.7 | 56.8 |
| Chromosome number judged to be 2n=16 | | | | | | |
| Manchuria: Moukden (TI) | 30 | 22.8 | 21.7 | 30 | 49.0 | 43.8 |
| Korea: Samgamyun (TI) | 30 | 22.0 | 22.6 | 30 | 56.7 | 44.2 |
| Korea: Inchun (TI) | 60 | 24.0 | 21.8 | 30 | 52.0 | 37.8 |
| Korea: Mt. Machonlyong (TI) | 30 | 23.4 | 22.5 | | | |
| Korea: Ansungli (TI) | 30 | 21.1 | 20.6 | | | |
| Siberia: Altai (GH) | 60 | 23.0 | 22.7 | 60 | 46.0 | 35.5 |
| Hungary: Mt. Tatra (TI) | 55 | 21.7 | 20.6 | 60 | 48.0 | 37.9 |
| Average | | 22.6 | 21.8 | | 50.3 | 39.8 |

southern part of Asia may be tetraploid (Fig. 1). From Himalayas, *C. foetida* has been reported by many authors: Hooker, J. D. (1872), Duthie (1906), Bamber (1916), Collett (1921), Hara (1966, the specimens from Sikkim and Nepal were examined in this study), etc. and Wallich (1930) as *Actaea frigida*, and Royle (1833-34) as *Cimicifuga frigida*. Judged from the present study, all these plants described from Himalayas may be tetraploid. They were recorded from Burma and Yunnan by Finet et Gagnepain (1904), Handel-Mazzetti (1931) etc., and are also considered to be tetraploid. On the other hand, *C. foetida* described from Siberia, Manchuria, Korea, or Europe by many authors may be diploid. A border line which separates diploid from tetraploid is considered to exist in the middle part of China. In the present study, however, the border-line was unable to draw from the lack of material from middle China.

I express my most cordinal thanks to Prof. H. Hara, the University of Tokyo, for his kind guidance and suggestions given throughout the course of this study. For the living plants from Bhutan, I am greatly indebted to Dr. H. Kanai and Mr. H. Ohashi, the members of the third Botanical Expeditions to Eastern Himalaya by the University of Tokyo in 1967. Many thanks are also due to the curator of Harvard University Herbaria for the loan of the specimens from Yunnan, China, and to Prof. S. Kitamura of the Kyoto University for kindly showing me the specimens from Bhutan.

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1967 年東京大学東部ヒマラヤ植物調査隊の金井，及び大橋両氏によって Bhutan の Thimphu から 3 株の *Cimicifuga foetida* (コウライショウマ) が採集され日本へ送られた。早速染色体数をしらべてみたところ 2n=32 であった。これまでの報告では，*C. foetida* は 2n=16 (Langlet 1927, '32, Lewitsky 1941)，及びすべての *Cimicifuga* 属の染色体数も 2n=16 であり 2n=32 は初めてである。染色体数と核型から 2n=16 は二倍体，2n=32 は四倍体と考えられるが，この二倍体と四倍体が外部形態の上で，又分布の上でどの様な関係にあるかを検討してみた。生材料はブータンのもの以外は入手出来なかったので標本を用いた。

*C. foetida* の分布はシベリア南部，朝鮮，満州，中国北部と西部，ヒマラヤ及び中部ヨーロッパの広い地域であるが日本には産しない。外部形態は非常に変異が多く特に花弁の形や植物体全体の毛の有無などについては変異がはげしい。しかしこの様な変異はどの地域内にも現れており，地域的な傾向がみられるわけではない。またブータンの生の植物については，東京に 2 株と軽井沢に 1 株栽培しており，東京の 1 株には昨年の夏 (1968) つぼみがついたが花が開かずに枯れてしまったため花の形態は観察していない。ここでは花粉粒及び気孔の大きさという点に注目して測定したところかなり明確な変異の傾向が現れた。花粉粒は幾瀬法によるプレパラートを作って観察し，気孔は孔辺細胞を含む長さと幅について，同程度に開孔していると思われるものを選んで測定した。ただし測定にあたっては花粉については 14 枚，気孔については 13 枚の標本から各々 30-60 個ずつしらべた。その結果，ヒマラヤ，ビルマ，中国雲南の植物は花粉，気孔ともに，アジア北部の朝鮮，満州，アルタイ及びヨーロッパのものよりかなり大きいのである。一般に気孔や花粉の大きさと倍数性とは関連が深いと思われるが，ヒマラヤ・ブータン産の植物が四倍体であり，ヨーロッパ産のものが二倍体と報告されていることを考えあわせてみると，おそらくヒマラヤ—ビルマ—雲南の一帯に産するものは四倍体であり，北部の朝鮮—満州，シベリア及びヨーロッパ産は二倍体であろうと推測される。そして二倍体と四倍体の境界は中国の中部にあると思われるのだが，今回の研究ではそのはっきりした境界を知ることは不可能であった。